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RESULTS OF USSR DISCUSSION ON SELECTION OF MICROORGANISMS

/The following is the complete text of an editorial that appeared in the June 1952 issue of the periodical Mikrobiologiya./

One of the distinguishing traits of modern technical microbiology is extensive utilization of various microorganisms. The number of biochemical branches of production increases with each decade; new branches of industry are entirely based on the utilization of microorganisms. It is enough to point to the antibiotics, enzymes, and vitamin industries, adoption of new fermentation techniques, etc. In some cases, as, for example, production of "agaric malt" in the fermentation industry, microrganisms not only successfully compete with the higher plants, but supplant them, because cultivation of microorganisms is economically more expedient. All this makes demands upon general and technical microbiology. These demands are connected with the search for new, practical, valuable forms of microbes and with the improvement of the nature of cultures that are already being utilized.

In contrast with the selection of higher plants, the selection of microorganisms began to develop considerably later and has not yet developed as a separate branch of science. The number of investigations devoted to the selection of microbes is comparatively small, while a large amount of factual material has been accumulated on the modifiability of microorganisms. This material is predominantly of a descriptive and theoretical character. Development of Michurin's teachings in microbiology is firmly connected with the creation of new, valuable stains of microorganisms that are needed by the national economy

In connection with all this, Mikrobiologiya launched a discussion on the problems of selection. It is necessary to clarify just what modern general and technical microbiology has at its disposal that enables it to control changes in the nature of microbes and then evaluate, on the basis of this discussion, various approaches and methods. The article by V. I. Kudryavtsev

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entitled "Continuous Selection of Microorganisms From Industry" was published as an initial article of this discussion, together with an editorial which contained an appeal to all Soviet microbiologists who are interested in the problem of selection, asking them to participate in the discussion which had been launched.

Fourteen additional articles were published in this journal. The articles in question discuss the problems and methods of the selection of microorganisms. Thirteen articles were written by microbiologists who work at specialized branch scientific-research institutes (otraslevyye instituty), while one was written by a man who is connected with the Academy of Sciences USSR. Representatives of various branches of industry, therefore, took part in the discussion.

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V. I. Kudryavtsev's article was devoted to the selection of yeasts; the majority of articles received by the editorial office, therefore, also had to do with the selection of yeasts. Only two articles were devoted to the selection of bacteria and two others were on the subject of valuable cultures of mold fungi. It is regrettable, therefore, that less light was thrown on selective work dealing with bacteria and mold fungi than on the selection of yeasts. Almost all authors who had their articles published emphasized that the discussion of this problem was timely and pointed out the necessity for organization of similar discussions in the future.

Before analyzing various opinions that have been expressed in the articles dealing with the selection of microorganisms, it is necessary to give a brief resume of the contents of V. I. Kudryavtsev's article. He thinks that during the past decades, as a consequence of preservation of standard cultures and the fact that industrial microbiology ceased to conduct a systematic selection of the more active forms from production, weakening of standard strains of fermentation microorganisms takes place. He suggests, therefore, that to change microorganisms in a manner we desired, it is necessary to carry on a continual selection from production of more active cultures and utilize them in practice subsequently to that. Of course, to familiarize themselves with the contents of the article, the readers must turn to the original text.

Positive Sides of the Method of Continual Selection From Production

Kudryavtsev's thesis that some fermentation microorganisms appeared as a result of unintentional selection by man and were developed by means of the production method is quite correct. In the first place, this has to do with cultured yeasts, which possess a number of physiological peculiarities that confirm this manner of their formation.

Many Soviet and foreign scholars emphasize the capacity of Saccharomyces cerevisiae to ferment certain dextrins. This is one of the characteristics that distinguishes cultured Saccharomyces from other species, particularly Saccharomyces ellipsoideus.

It is very probable that during centuries, or possibly millenniums, spontaneous selection that took place in industries led to the appearance of domesticated, actively fermenting forms. Kudryavtsev explains by such selection the presence of maltose in yeast cells. Stressing that maltose is rarely found in plant tissues, the author draws the conclusion therefrom that maltose is rarely found in nature. It is hardly possible to agree with that conclusion, because it is necessary to proceed not from statistical biochemistry, but from the dynamics of biochemical conversion which are observed in nature. It is generally known that a considerable amount of starch contained in plants decomposes in nature because of the action of microorganisms which contain amylase; as a result of this process maltose is formed. This explains why the enzyme maltase is so widely distributed among wild soil fungi and bacteria which cannot be counted among cultural, domesticated forms.

It is more correct to assume that as a result of unintentional selection, forms have developed which possess a great capacity for alcoholic fermentation. Man, in creating conditions favorable for alcohol fermentation, obtained forms which produce the maximum amount of alcohol in the least amount of time.

As the principal advantage of the continual method of selection of cultures from production, we must regard the fact that microorganisms utilized in production will always be adapted to the conditions of production. Furthermore, they will be more perfectly adapted to all exigencies of production than preserved standard strains. Fulfilling a definite function, all enzyme systems of the microorganism will work most actively and produce the maximum output of desirable products. Such influence of the conditions of production will be particularly sharply pronounced when the difference between existence in a repository and in production is particularly great.

It is impossible to agree with the opinion of L. I. Chekan, who thinks that during continuous reproduction of microbes used in industry there naturally must appear a weakening of the culture. Such weakening is possible, but does not necessarily occur.

Although correctly stressing the advantages of continuous selection from production, Kudryavtsev overestimates the achievements of foreign manufacturers. Speaking about the brewer Yakobson, who during a number of years worked with the same culture of Saccharomyces, Kudryavtsev writes: "Not a single modern brewery can brag of such results, even though the technical equipment and the sanitary-hygienic conditions at modern breweries are much superior to those existing earlier."

A number of microbiologists voiced their disagreement with this statement in the pages of Mikrobiologiya. Many of those who participated in the discussion quite correctly stressed the achievements of Russian microbiology in the realm of selection of microorganisms obtained by the method of picking cultures from industrial production. A number of examples were given by Russian and Soviet microbiologists on obtaining active cultures of Saccharomyces, which cultures are utilized now in the alcohol industry, wine manufacturing, yeast production, and brewing. There were also references to a number of cultures of vinegar and lactic acid bacteria, which were obtained by the method of selection from industry and which found wide practical application.

Information on the authors who obtained these cultures and the names of the microorganisms and the concrete fields of their use was presented in detail in the articles written by E. I. Kvasnikov, I. Ya. Veselov, L. I. Chekan, K. V. Kosikov, O. M. Silishchenskaya, and others.

It is regrettable that Kudryavtsev has not sufficiently utilized this material to stress the possibilities of the method of selection from industry. His article also did not contain the results of his personal investigations in that direction.

The merits of this method were noted in the articles of I. M. Ryabchenko, N. I. Kayukova, S. S. Leshchinskaya, and G. I. Mosiashvili, who reported interesting results obtained by using the method under discussion.

It is impossible to agree with the opinion of K. V. Kosikov, who participated in the discussion and who considered that continuous selection from industry deserves practically no attention.

Thus, we see that continuous selection from industry is a method which has a definite field of utilization and distinct possibilities, and may be recommended as a method of selection.

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Continuous Selection From Industry As the Leading Method of Selection

Fields of Application of the Method

Present-day technology of the biochemical production is so diverse that it is practically impossible to offer general recommendations in the field of selection. It is necessary to individualize thoroughly the methods of approach, proceeding from the concrete conditions of the present production and the peculiarities of microorganisms utilized in it. The field of application of continuous selection from industry is restricted, because in some cases this method cannot be employed at all. There are a number of productions in which prolonged use of the same culture produces weakening, but not its intensification, as desired in continuous selection. It is obvious that this reduction of activity is due to insufficient knowledge about the fine points of the nutritional physiology or microorganisms and of those conditions which are necessary for the development of a given microbe, irrespective of the influence of extraneous microorganisms producing infection. Weakening of bacterial cultures which decompose pectin substances is well known. In reproduction of lactic acid bacteria and others used in acetone-butyl alcohol production, degeneration of cultures is encountered.

In some biochemical industries, use is made of fresh standard cultures in each technological cycle. In these instances, the distinct strain remains in production for a short period of time, and the maximum formation of the useful product occurs during the second phase of the production process, when the culture is subjected to lysis and becomes unfit for selection. Here it is difficult to expect considerable changes as far as any increase of the activity of the culture is concerned. It is also necessary to take into consideration that resistance to extraneous microorganisms (i.e., to infection) is a property which does not always arise, because many industrial processes are conducted under sterile conditions that exclude the possibility of the development of other organisms. This refers to the antibiotics industry, the enzyme industry, and others. However, even when the conditions of manufacture are "nonsterile," improvement of characteristics of the culture does not necessarily take place. Therefore, the observations made by L. I. Chekan and E. I. Kvasnikov are correct. Their observations were that isolation of a culture from a production process in which extraneous microorganisms are present may lead not only to the desired reinforcement of the functions of the production culture, but also to its weakening as a result of the action of extraneous microorganisms.

Possibilities of the Method

The method of continuous selection from production is a continuation of that lengthy process which created the forms cultivated at present. It is necessary, however, to take into consideration the fact that the development of cultivated forms took place during an extremely long period of time. To expect considerable changes in the physiology of a microbe during short periods of time is therefore hardly possible

O. M. Siliahchenskaya points out in her article that out of 500 cultures of Saccharomycetes taken from industry, there was not a single one which differed considerably in respect to its activity from the initial culture. Analysis of figures quoted in the article of S. S. Leshchinskaya published in Mikrobiologiya also refers to comparatively insignificant changes.

A positive effect, therefore, may be obtained during protracted periods of time by accumulation of those minor changes which are possible during production

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It is necessary to add, moreover, that under present-day conditions of production strict observation of set procedures is of great significance. The more highly standardized the conditions under which the microorganism exists, the better the chance of obtaining a continually high output. Any kind of significant deviation from the established conditions leads to a lowering of output.

In this connection, the question naturally arises whether it is possible that considerable impairment of the heredity of a microorganism may occur when conditions of existence of the microorganism are constant. The answer is negative. Only by reason of effects produced by unusual conditions, and because of considerable impairment to heredity, may new cultures of microbes with changed heredity appear. To assume that under conditions which correspond to the requirements of an organism, greatly changed forms constantly appear, signifies adherence to the idea of mutationism. In some types of biochemical production, the composition of the medium and the conditions of cultivation are standardized to such a degree that no grounds exist for talking about any great impairment of heredity. This applies particularly to the cultivation of fungi or of bacteria in synthetic media of a constant and definite composition. But even in those industries which are based on utilization of vegetable raw material, there exist uniform technological procedures for obtaining wort. It is hardly correct, therefore, to speak about the possibility of adaptation of a microbe to conditions which exist at certain industrial plants. It is very evident that the more diverse the fermented material, and the more varied the conditions of production that are connected with the local climatic and other factors, the greater are the chances of obtaining from industry, by selection, forms which differ from the original culture.

This situation is confirmed partially by the fact that particularly successful selection of Saccharomycetes from industry is made in wine-making, baking of bread, and vinegar manufacturing, where many of the processes are not standardized.

As far as other industries are concerned, the conditions are different. All that has been stated above makes it clear why it is that when cultures are taken from industry, efforts to obtain cultures with greatly changed characteristics usually do not meet with success.

Passive and Active Methods of Selection

Characteristic of present-day creative Darwinism is production of active changes in the nature of microorganisms and subsequent practical utilization of the resulting microorganisms. It is completely justified to refer to this method as an active method, because the experimenter by changing the conditions of existence of microorganisms may be said to mold the forms that are needed.

In contrast to this, the selection from industry is a passive method, which is based on changes resulting from modification of conditions encountered in industry.

The theoretical foundations of present-day biochemical industry exclude the possibility of changes in production methods and thereby exclude the possibility of any great modification in the nature of microorganisms. In contrast to this, the experimenter in the laboratory may create any kind of conditions, and possibilities available to him in this connection are almost unlimited. He may create the necessary concentration of carbohydrate within the medium, exert control over breeding of microorganisms in the presence of any poison or antiseptic, adapt the culture of the microorganism to low or high temperatures, high concentrations of alcohol, various antibiotics, etc.

As a concrete example, it is necessary to point to the adaptation of Saccharomycetes to considerable alkalinity or acidity of the medium, and to the adaptation of mold fungi to antiseptics that may present practical interest in connection with surface breeding of fungi which are used in obtaining enzyme preparations.

It is known that increases in the yield obtained in acetone-butanol fermentation are limited by the sensitivity of acetone-butyl alcohol bacteria to accumulated butanol in the medium. In connection with this, it is theoretically possible that adjustment of bacteria to butanol in laboratories will permit later employment of more concentrated mash.

As examples of such preliminary experimental changes in heredity of microorganisms and subsequent utilization of a new variety under new conditions of production, one can cite the development of cultures of microbes which are used in determining vitamins and amino acids as well as the development of an active variety of Penicillium chrysogenum. According to information gathered from publications, a productive race 70 times more active than the original one was obtained from the wild culture of Penicillium chrysogenum. The newly obtained variety is a form which demands altogether different conditions for more complete manifestation of all its new properties, particularly cultivation in depth, addition of precursors of penicillin to the medium, etc.

It is quite obvious that repeated isolation of wild forms of penicillin from production would not lead to the appearance of a form having such high activity.

The principles of directed intensification of biochemical properties of microorganisms are less developed. Of particular significance here is a profound study of the metabolism of microbes, appropriate management of which must lead to needed changes in the microorganism. The study of phasic development of microbes is just as important, because it has a direct bearing on the derivation of new forms and the setting up of correct experiments in the field of directed modification. The significance of phasic development was very correctly brought out in Z. E. Bekker's article, which was published in connection with the discussion.

The microbiologist, having obtained a modified culture, alters the type of production in conformance with the demands of the organism. Under present-day research techniques, the experimenter may to a considerable degree simulate in the laboratory the conditions existing in industries. However, this does not mean that later on, before the culture is accepted, the necessity may not arise for supplementary adaptation of the microbe to industrial conditions, particularly as far as cultivation of large quantities is concerned. The method of selection from industry. therefore, must be classified as a passive method, while modification of the nature of microbes and subsequent changes in production itself, which corresponds to the new demands of the active form of the microorganism, must be regarded as active methods of selection.

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Comparative Evaluation of Existing Selection Methods

During the past 2 years, two conferences were held at which problems connected with the selection of microbes were discussed. One was the All-Union Conference on the Directed Modification and Selection of Microorganisms, held in 1951. It was conducted by the Institute of Microbiology, Academy of Sciences USSR. The other conference was called by the "Magach" Institute of Viniculture, and was held in 1950 at Yalta. As a result of discussions conducted at these conferences, a number of problems in connection with the selection of microorganisms were clarified, and an appraisal was made of various existing methods. It was pointed out in the resolution passed by the All-Union Conference on Directed Modification and Selection that directed modification must be adopted as the guiding principle by creative Soviet Darwinism and must become the leading working method for the alteration of the nature of microorganisms. This directed modification is attained either by cultivating the microorganisms under changed conditions of existence or by conferring new properties through vegetative hybridization. Although the latter method did produce interesting results with reference to the intestinal group of bacteria, it is necessary to clarify the perspectives of the method in question as far as its application to the selection of microbes is concerned.

In the same category of directed modifications must be placed those changes in metabolism and subsequent morphological changes which arise in microorganisms under the condition of laboratory cultivation. These changes, including also the so-called dissociation, correspond to the existing living conditions of microbes and represent a particular case of directed modification.

Moreover, the conference stressed that application of strongly acting factors cannot be the leading method of selection. However, if the forms which are obtained by this method and which have less vitality and a distorted metabolism find practical employment, then this method should also be used. Finally, the possibility was admitted that microorganisms which have a sexual process may furnish valuable forms by the way of sexual hybridization. The conference also noted the importance of investigating the role which /non-cellular/ living matter and formation of filterable forms of bacteria play in the development and modification of bacteria.

All five methods described represent experimental methods which are connected with the changing of microorganisms under laboratory conditions.

At the same time, the resolution stated that there is a need to continue search for useful forms in nature and to bring about selection of microorganisms by selection from industry. Neither of these two methods involves active alteration of the nature of microorganisms that is carried out at the will of the experimenter. He only uses something that is supplied by nature on the one hand and by industry on the other.

Standard Cultures

A few microbiologists who participated in the discussion which was conducted in the pages of this periodical have correctly pointed out that elimination of standard cultures of microorganisms kept in stock at repositories does not serve any useful purpose.

Of course, it is impossible to deny that microorganisms, which are transferred from the soil, water, vegetable raw material, etc., into surroundings encountered in repositories for stock cultures, are subjected to exceptionally sharp changes, which in turn produce changes in metabolism. S. N. Vinogradskiy and other microbiologists have repeatedly pointed out this situation. It is

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quite natural that microorganisms which are accustomed to exist, for example, in water containing a small concentration of organic substances sharply change their metabolism when transferred to meat peptone bouillon. This leads to considerable changes in the characteristics of the microorganism. The cultures of bacteria, on being subjected to stock repository conditions, begin to lose their natural characteristics and become stock repository forms. This process represents the first stage of domestication.

Apparently, such changes are characteristic for wild forms of microbes taken from nature.

As far as cultures of Saccharomyces are concerned, the oldest cultural strains sometimes completely preserve their vigor during prolonged periods of existence as standard cultures. Thus, according to data of O. M. Silishchenskaya, the varieties of Saccharomyces II.M; YA.XII, and others, did not lose their vigor during the 20 years that they were kept in stock repositories. Alcohol produced in those factories where pure cultures of Saccharomyces were used was 1.9% higher than in industries where Saccharomyces was used without replacement.

It is obvious that an up-to-date stock repository for cultures that are valuable for practical use must not be used only as a storehouse. Strenuous scientific experimental work must be carried out in it. The tasks of this work consist primarily of the following:

1. Development of principles for further selection by means of directed modification.

2. Study of methods for the preservation of valuable production characteristics. It is evident that conditions must be created in stock repositories for cultures that would duplicate those under which the microorganisms function in industry. This refers to osmophilic, acidophilic, thermophilic, psychrophilic, and other varieties of bacteria, yeasts, and fungi. Transplanted cultures under ordinary conditions will of course lose the characteristics enumerated above. This, however, does not mean that in some cases conditions of cultivation in stock repositories cannot be different from those under industrial conditions. It is well known that industrial cultures of microorganisms can survive a single unfavorable action of a physical or chemical character and still produce good yields. Cultivation of microbes under constant exposure to any such action results in their degeneration, however. For instance, this refers to Saccharomyces which produce alcoholic fermentation in strongly alkaline media.

As another example, one may point to microorganisms from which antibiotics are produced by industry: they multiply in media which contain precursors of antibiotics and these precursors are toxic to the microorganisms producing them. However, in stock repositories the microorganisms exist in media which are free of toxic precursor . Theoretically, it is possible to obtain forms which are adapted to toxic precursors and it is reasonable to assume that such adaptation will be accompanied by an increased biosynthesis of antibiotics. Judging from published data, however, stock repository cultures up to the present have been preserved in media which do not contain precursors of antibiotics.

3. Research must also be conducted at stock repositories that would explain the reasons for the degeneration of cultures if such degeneration is observed.

4. There is no doubt that it is necessary to conduct investigations at stock repositories of cultures with the view of systematizing valuable cultures of microorganisms. It is necessary to take into consideration that cultures kept at stock repositories in some instances lose characteristics that are valuable from the standpoint of practical uses to which the cultures are put.

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This leads to the necessity, even when all precautions are observed after a vigorous culture has been isolated, to lay in immediately a store of seeding material for a protracted period of time. With that purpose in mind, standard material containing spores of bacteria is prepared. Another method is to subject cultures of microorganisms to lyophilic drying and then store them in that state. Storage of standard cultures in stock repositories should be retained, but the methods of storing the cultures ought to be altered. Furnishing various branches of industry with cultures from central repositories that are adequately manned by scientifically qualified personnel is the most reliable method of assuring effective work at industrial enterprises.

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